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Measuring Copper in Seawater—An Automated Detection of Copper Binding Capacity Final Report of SERDP SEED 1266

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**Measuring Copper in Seawater –
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Final Report of SERDP SEED 1266**

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Abstract

The mechanism for the detection of copper by ion selective electrodes in seawater has been examined. Contrary to common perception, the direct detection of copper with ion selective electrodes is unlikely to be possible due to the low levels of uncomplexed copper present. Instead, it is proposed that the ion selective electrodes measure the activity of naturally-occurring binding ligands for copper and thereby provide an indirect determination of uncomplexed copper, which is the species most likely to be toxic to organisms. A potentially automated system is described to measure the complexing ability of the seawater system for copper and other metals. This system can allow the determination of excess binding capability for the water system and thereby predict if a water body can absorb extra toxic metals.

Introduction

Copper in bays and estuaries can come from many sources. The Navy uses large quantities of copper based paints as antifouling agents on ship bottoms. Some of this copper is constantly leaching into the environment. Also, large amounts of copper are released when ship hulls are scrubbed to remove barnacles and biofilms that greatly increase fuel consumption. Other sources of copper can come from dredging operations, or even ship travel, which may reintroduce copper from the sediment where it is presumably not as bioavailable. All sources of copper must be monitored to mitigate the impact to the environment and to account for the relative impacts of the sources. In estuary environments, copper is toxic in low concentrations and therefore regulated by the EPA. If the regulatory level is exceeded, then drastic actions may be required, including the reduction or prevention of use of copper anti-fouling paints.

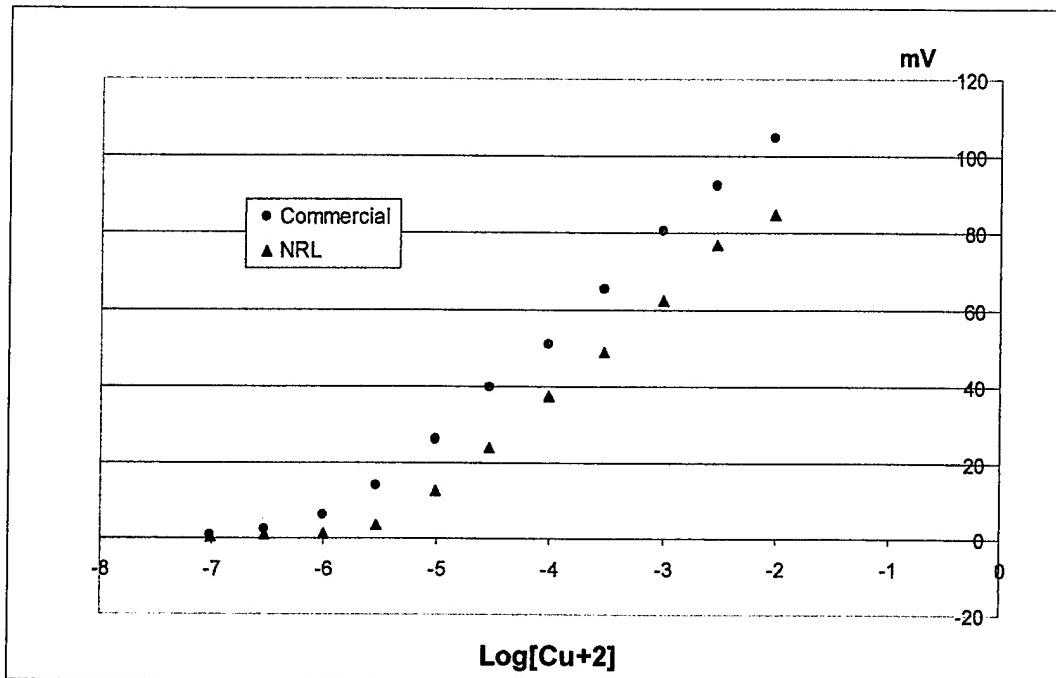
Total copper in seawater varies widely depending on the sources and water exchange with the ocean reservoir. In San Diego harbor it can sometime exceed the EPA action level of 3 ppb or $4.7 \times 10^{-8} M$.¹ Most of this copper is bound to organic and inorganic ligands and is not available for interaction with bioorganisms. The free copper in seawater is typically 10^{-11} to $10^{-12} M$. Several analytical techniques including stripping voltammetry, dialysis and concentration, mass spectrometry, and ion selective electrodes (ISE) have been used to monitor copper in water. For convenience and speed of analysis, ion selective electrodes cannot be surpassed. ISEs respond to only free copper not the total copper present. Unfortunately, the free copper level in seawater is several orders of magnitude below the Limit of Detection (LOD) measured in the laboratory for copper ion selective electrodes. Nevertheless, a procedure has been developed to provide this measurement.¹ This procedure has also been extended to the measurement of free Fe(III) in seawater.² The use of copper ion selective electrodes in seawater is not without criticism because of the interference observed with chloride. Some of this criticism has been addressed.³

This paper reviews the technique employed to measure copper in seawater using ion selective electrodes. It then discusses an alternative mechanism for the observed results based on the measurements of free ligands for copper rather than copper itself. Finally, it describes a system for the measurement of ligand binding capability based on electrochemical generation of metal ions. This system could be miniaturized and automated.

Results and Discussion

Copper ion selective electrodes are based on the jalpaite membrane structure (nominally $Cu_2S \cdot 3Ag_2S$). This is a solid state (pressed pellet) type of ion selective electrode and can detect copper(II) down to $10^{-10} M$ (0.006 ppb).^{4,5} This electrode has been commercialized⁶ and used in fresh water studies.^{7,8} When the jalpaite electrodes are calibrated in either distilled water or artificial seawater,⁹ similar calibration curves to what is found at NRL are observed, *i.e.* the electrodes have a limit of detection (LOD) of about $10^{-6} M$ (judging from the published graph and using the standard of 8.7 mV ($29 \text{ mV} \cdot \log(2)$) deviation from a straight line)). This level is not the ultimate LOD, because the calibration curve deviates from a straight line, and it is still above the blanks for about another two decades of concentration. A calibration curve in distilled water is shown in Figure 1.

Figure 1 – Calibration curves for a commercial, copper electrode (Cole-Palmer) and one prepared at NRL. Both the commercial and NRL developed electrodes had erratic results between electrode preparations. The LOD for the commercial electrode was about 7.5e-7M and that for the NRL electrode was about 1.8e-6M.



Because the free copper in seawater is so low, a procedure has been developed for use of the copper ion selective electrode.¹ First, the copper selective electrode is calibrated in two metal-ion buffers (chelated copper that provides a small but constant source of copper) as follows: (1) A solution is prepared containing 2×10^{-4} M Cu and 10^{-3} M ethylenediamine (EDA) in artificial seawater. The solution is adjusted to a pH between 8 and 8.3 using dilute HCl. The free copper is calculated based on the measured pH using an extensive table showing the free copper concentration vs. pH for the EDA-Cu system based solely on calculations (see Table 1 for a brief summary). (2) A second solution of 2×10^{-4} M Cu and 10^{-3} M glycine in seawater is prepared and adjusted to the same pH as the EDA-Cu buffer using sodium hydroxide. Again, calculations are preformed showing the free copper vs. pH for this system. To calibrate the electrode, it is placed in the glycine buffer and allowed to stabilize. This can take hours. During this time, the glycine buffer is changed three times. After stabilization, a mV reading is taken. The electrode is then placed in the EDA buffer, allowed to stabilize, the buffer changed three times, and another mV reading taken. The mV difference should reflect the difference in the calculated free copper (from Table 1) and the slope of the ion selective electrode (assumed to be 29 mV/decade). Thus, for a pH 8 solution, the free copper difference is 3.954 (log values) and the observed mV difference should be approximately 104 mV.

Table 1 – Calculated concentrations of free copper ions in various buffers. Buffers are composed of 10^{-3} M chelating agent, 2×10^{-4} M copper in artificial seawater. Note: the slopes of the EDA and glycine lines are not the same in a plot of pCu vs. pH. Therefore, the difference in free copper and hence the mV separation will vary depending on the pH. Data are obtained from SPAWAR.¹

pH	pCu in EDA buffer	pCu in Glycine buffer
8.00	12.885	9.401
8.05	12.998	9.49
8.10	13.112	9.58
8.15	13.225	9.67
8.20	13.339	9.76
8.25	13.453	9.849

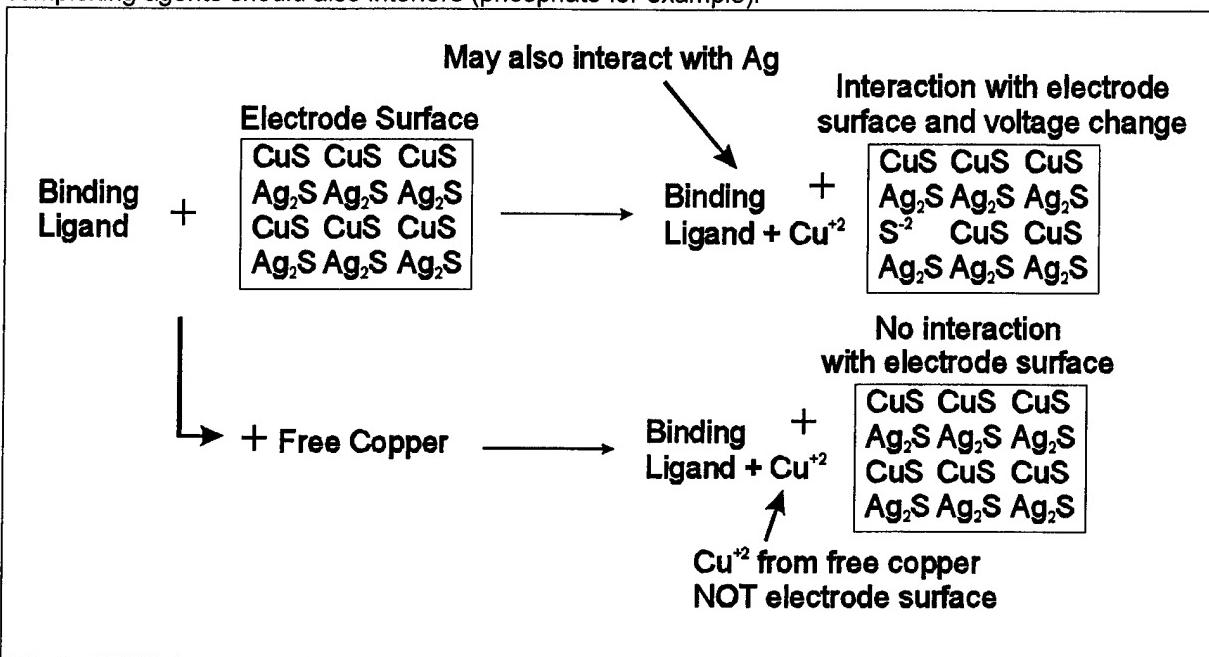
After calibration, the electrode is placed in the seawater sample and allowed to equilibrate for some time. The sample is replaced with a fresh sample and the mV reading taken when a stable voltage is reached. The free copper in the seawater is calculated from a two-point line obtained from the above calibration. Trials off a dock in San Diego harbor showed a constant response for copper indicating little or no change in concentration vs. time. This procedure has also been used in a flowing system, where the electrode is initially calibrated and data are taken for several days.

The values obtained from the ion selective electrode procedure appear to agree with values obtained from other, more sophisticated analytical procedures.³ We then reach a dilemma. How can measurements three to four orders of magnitude BELOW the LOD be made? This problem has been recognized before, but no adequate explanation has been provided. The literature on copper ISEs is very complex and conflicting. It is likely that all the technical literature on this point is WRONG regarding the mechanism of detecting free copper in seawater but likely RIGHT in the outcome.

An alternative explanation can be proposed as follows: The free copper is NOT being measured. Instead the ion selective electrode is reacting to the natural chelating agents in a manner reflective of the binding constant of the chelating agent to copper IN THE CRYSTAL LATTICE of the solid-state electrode and at the surface. The copper in seawater is merely binding the chelating agent and preventing it from interacting with the electrode. An example of this scheme is shown in Figure 2. Alternatively, the chelating agent (EDTA in Figure 2) may be adsorbing on the surface of the electrode rather than removing a copper ion from the crystal lattice. Adsorbed negative ions would cause a decrease in potential generated by the electrode. The two mechanisms could possibly be distinguished employing a neutral (non-charged) chelator for copper, similar in structure to a crown ether. If copper were being removed from the lattice then the neutral chelator would still show a negative potential affect. If the chelator was complexing the copper on the surface, then the neutral chelator should show no effect. Experiments to better define the mechanism are being planned.

Laboratory experiments show a similar effect. For example, when EDTA is added to a copper ion-selective electrode in distilled water, a large change in voltage in a negative direction is observed (Figure 3). This is frequently observed in the literature but the explanation has been that the system is responding to free copper rather than that the electrode is responding to removal of copper from the lattice by the chelating agent.¹⁰

Figure 2 – Scheme for the response of the copper ion-selective electrode to ligands. Note that the removal of the metal ions occurs at the surface and produces an electrical potential that inhibits removal of more metal ions. Unfortunately, this is hard to measure by surface techniques, such as XPS, because this equilibrium is only maintained in water due to charge balance considerations. The vacancy in the crystal lattice will best fit the ion removed rather than other charged species. The binding ligands may also interact with the silver ions in the crystal lattice of the ion-selective electrode. Thus, the response may not be only due to removal of copper. Additionally, the jalpaite crystal is known to have an interference with chloride, presumably by removing some silver from the lattice to form AgCl. Other silver complexing agents should also interfere (phosphate for example).



A clear test of the hypothesis shown in Figure 2 is difficult to design. If the hypothesis that the copper electrode responds to chelating agents is correct, then anything that competes with copper for the chelating agents should cause a response in the electrode. Basically, EDTA is being titrated. In the presence of EDTA, the copper electrode would respond to calcium (Figure 3) but not without EDTA (Figure 4). Near the equivalence point the voltage level is similar to the starting value in distilled water. After the equivalence point, no free EDTA is present so that the electrode no longer responds to the added metal ion. The small response is due to varying ion activities due to changing ionic strength. Calcium does not have as strong of affinity to EDTA as does copper (Table 2) so that the equivalence point is less sharp. On the other hand, nickel has a similar binding to EDTA as does copper and also causes a response for the copper electrode in the presence of EDTA but not in its absence. The response is somewhat sharper than calcium after the endpoint due to the higher binding (Figure 5).

Table 2 – Complexation constants for EDTA and several ions.

Ion	Log (complexation constant)
Cu^{+2}	18.80
Cu^{+1}	19.5 ¹¹
Ca^{+2}	10.69
Ni^{+2}	18.62
Fe^{+3}	25.1

Figure 3 – Response of commercial jalpaite electrode to EDTA and added calcium. Two NRL electrodes are shown for reference. The NRL electrodes were prepared as per Neto, *et al.*¹² However, X-ray powder diffraction showed that this preparation was clearly a mixture containing CuS and very little jalpaite (Figure 7). From distilled water (0-750 seconds), the electrodes were introduced to a 1×10^{-3} M EDTA solution (pH = 10). Then Ca^{+2} was added in increasing concentration. Note the large negative voltage change in the presence of EDTA and near return to original values when the EDTA is complexed by the calcium.

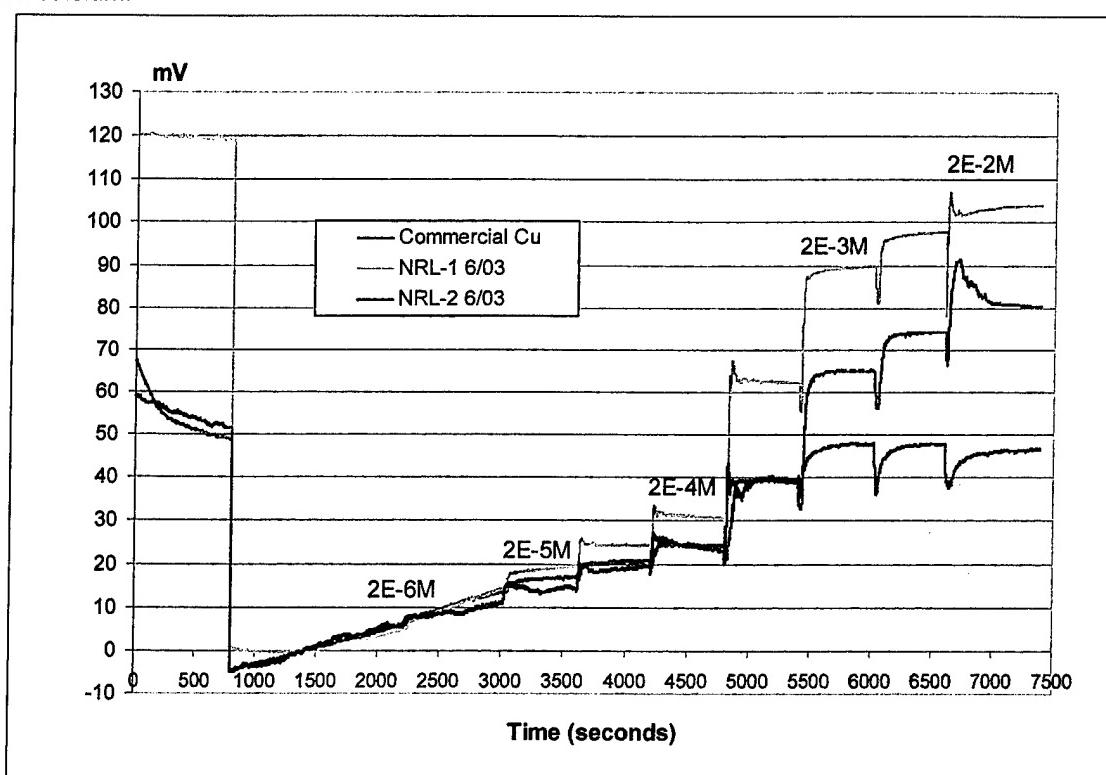


Figure 4 – Response of the three electrodes in Figure 3 to calcium in the absence of EDTA. Little response to calcium in the absence of EDTA is observed indicating that the electrodes in Figure 3 are not responding to the calcium additions.

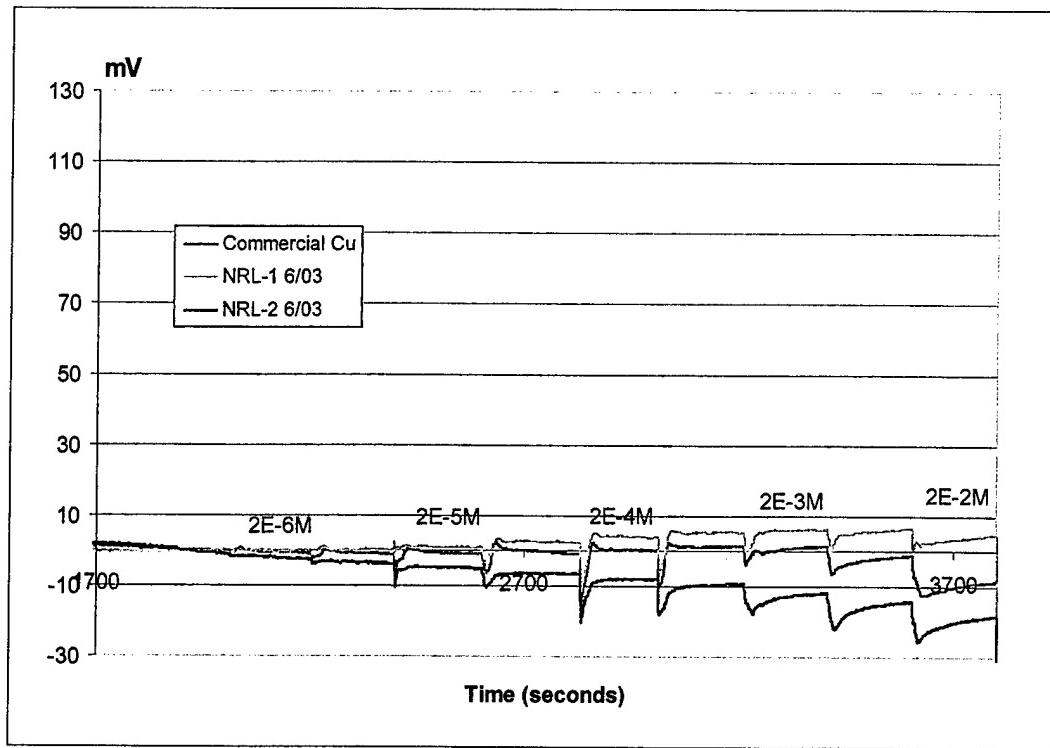
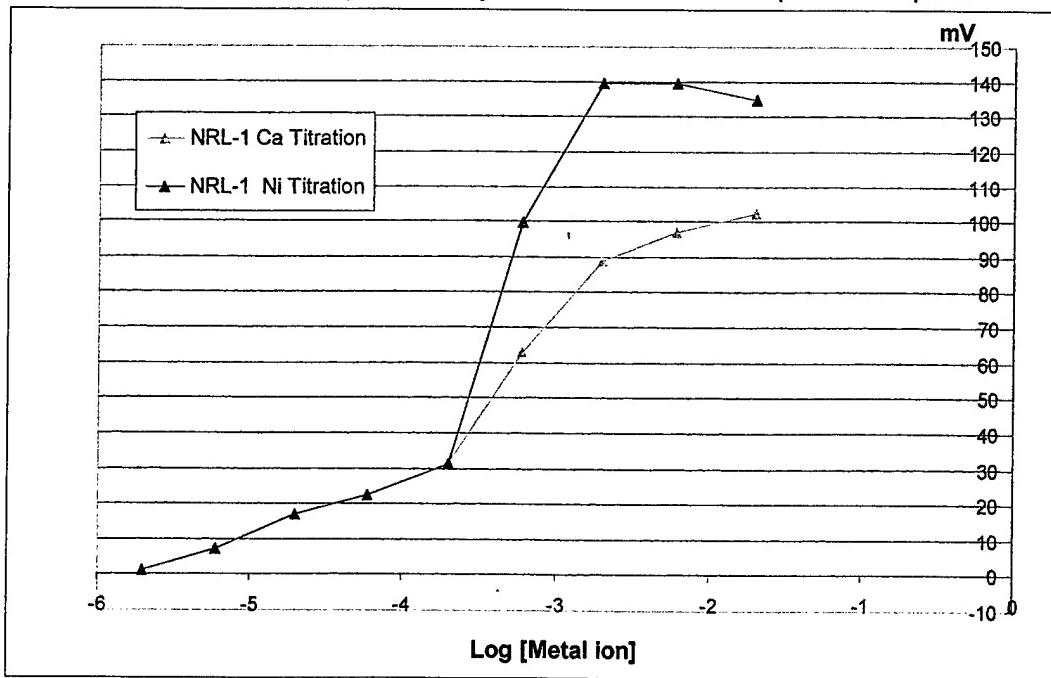


Figure 5 – Titration curve of electrode NRL-1 6/03 in EDTA with nickel and calcium. The nickel curve is sharper and returns to a higher mV level than does calcium because nickel has a higher and similar binding constant to copper whereas calcium is much lower (Table 2). The EDTA concentration was 1×10^{-3} M. The orange and blue plots overlay each other before the equivalence point.



An alternative explanation for the effect of chelating agents is in complexing free copper resulting from dissolution of the electrode surface. Complexation of the dissolved copper removes this competing ion from the crystal surface, presumably in the vicinity of the surface and at somewhat higher concentrations than the bulk solution. This explanation is difficult to reconcile with the solubility product of the electrode, which can be estimated to be $1.7 \times 10^{-16} M$.¹³ Additionally, in a flowing system, the sensitivity is not much better to that in a static system, which could imply fast kinetics to establishing an equilibrium or that the mechanism of dissolution of the membrane is incorrect. If the membrane dissolves rapidly, then in a flowing system, rapid erosion should occur because ions are not available to reprecipitate back onto the electrode. The surface of copper ion selective electrodes appears stable implying that membrane dissolution is slow, as would be estimated from solubility products.

The measurement of free copper at very low levels in seawater appears to be fortuitous. This system works in natural seawater (but not artificial seawater) because natural seawater also contains binding ligands that interact with copper and it appears that the copper ion selective electrode responds to copper binding ligands. In fact, the majority of the total copper in natural seawater is bound by these ligands and is not free to interact and be toxic to organisms.^{14,15,16} Thus, what is actually being measured in natural seawater is not free copper but FREE LIGAND CONCENTRATIONS. These are related to copper ONLY to the extent that natural seawater is a system where other ionic species are RELATIVELY CONSTANT in concentration. If these other ionic species were to increase, the ion-selective electrode would then have fewer ligands for interaction and would then respond as if free copper were present when it is some other metal (as for example did the calcium and nickel responses in Figures 4 and 5). Therefore, this system of measuring copper only works when the copper levels are high enough to be near the saturation of the natural ligands OR the other ionic species that may also interact with these ligands are very consistent in concentration over time. Erickson, *et al.*, has partially observed this experimentally.¹⁷ In their experiments with flathead minnows, if the hardness of water was increased, the toxicity of copper decreased initially but quickly leveled off. A continual decrease in toxicity would be expected if calcium/magnesium were competing for copper binding to the organism. In this case, the added calcium/magnesium is possibly releasing more copper from natural ligands and thereby counteracting some of the protective effect of the added ions. If one could generate a water system with zero copper yet have natural ligands present, the ion-selective electrode would respond as if the copper concentration were very low. However, if other metal ions were added to that system, then the ion-selective electrode would start to respond as if copper were present, when it is not. An example of such an artificial system (one using artificial ligands) is with EDTA, discussed above.

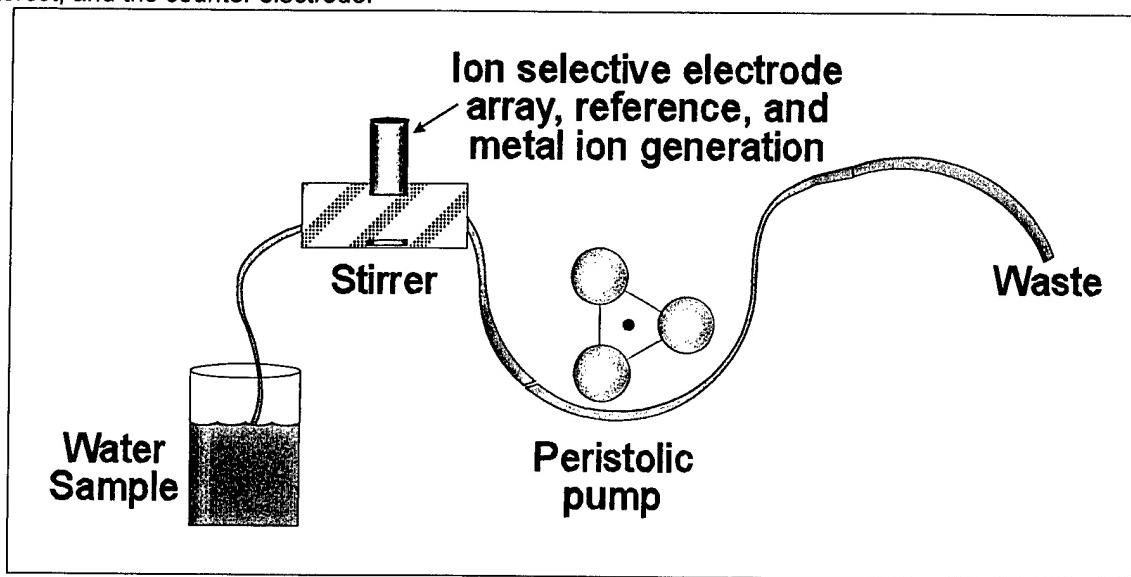
When used in seawater, the trends observed with copper ion selective electrodes are similar to the measurements using other procedures. However, the results are not exact because other metals may be present to interfere with the ligand binding. Additionally, the exchange between the ionic crystal, free copper, and natural ligands may be kinetically controlled. This is why measurements can take hours rather than the response time of seconds for most ion-selective electrode measurements and the lengthy time required for calibration of the electrode. In continuous flow systems, again it is fortuitous, that the ligands do not change their concentration rapidly over time so the ion-selective electrode would have a chance to adjust its potential to the changing ligand concentration.

It is becoming more accepted that the toxicity of copper is due to the free copper present rather than the total copper, which is what the EPA regulates.^{18,19} Thus, in estuaries, which have large amounts of organic matter that can complex copper, more copper can be accommodated without toxicity than in pristine water without the organic matter. Additionally, the binding

capacity of this organic matter for copper and some measure of the free copper present must be determined. If the binding or buffering capacity is low then that watershed would be more susceptible to copper pollution than one where the binding is much higher.

An interesting system could be designed to automatically measure both the ligand binding ability and the amount of ligands for copper. This system would be composed of a copper ion selective electrode and a copper wire that could be used to generate known amounts of copper electrochemically. A schematic of such a system is shown in Figure 6. Water would be introduced by the peristaltic pump into the electrode chamber and the free copper activity measured with the copper ion selective electrode (if the hypothesis described above is correct, the amount of ligands in the sample would be measured). This may be a continuous process. Periodically, the pump would be stopped and the stirrer started. Copper (or any other metal) would be generated electrochemically by galvanic corrosion of the appropriate wire. The pH, copper activity, and other ions would be constantly monitored. If the water had poor ligand binding capacity, then an immediate rise in the copper ion selective electrode readings should be observed. If the ligand binding were substantial, some time would elapse before the signal changed. This time would be proportional to the ligand binding and the amount of copper generated, which can be estimated from the current required. Monitoring of pH is necessary because copper binding may be pH sensitive. Binding to other metals of interest could be generated in an identical process by having other metallic wires present. Once the copper binding measurement was completed, the cell would be flushed for another measurement or continued background measurements.

Figure 6 – Schematic of an automated system for measuring free metals and their binding capacity. The array would contain both the ion selective electrodes (pH, Chloride, copper, etc.), the reference electrode for the potentiometric measurements, the metal wires for generating the metals of interest, and the counter electrode.



For testing of the system outlined in Figure 6, ion selective electrodes must be prepared and a potentiostat system designed. The descriptions of both approaches are outline below.

Preparation of copper detecting membranes

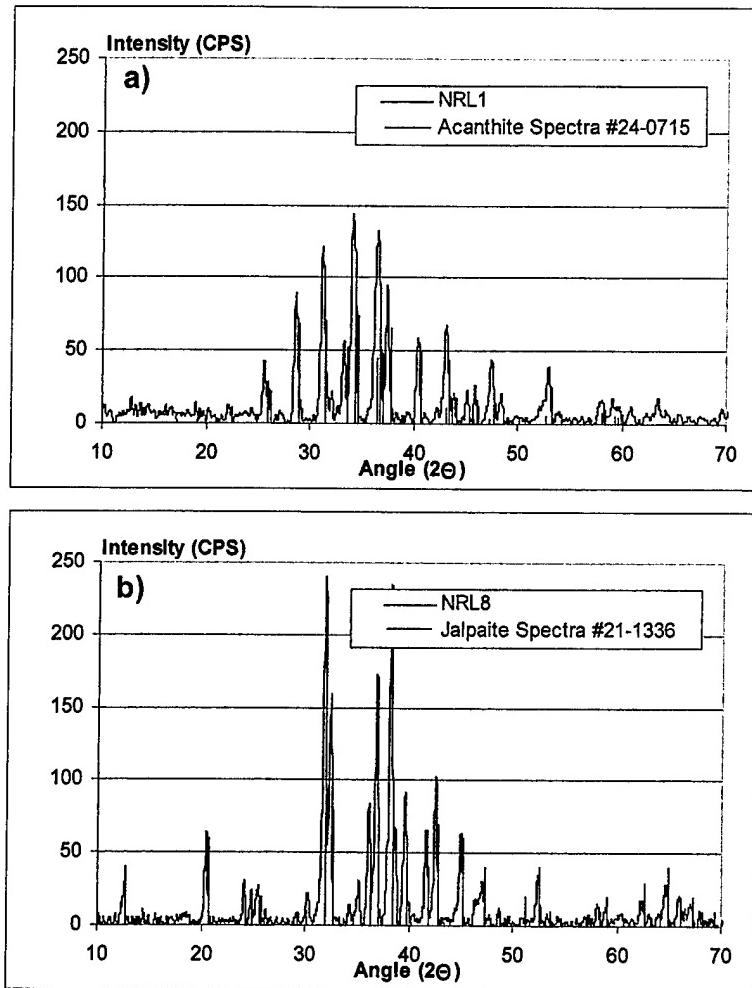
The literature discusses a large number of preparations of copper selective membranes.^{12,20,21,22,23,24} Most are based on the Cu₂S-Ag₂S system. This tertiary compound can be made with a number of phases depending on the exact preparation procedure. Many of these phases have been partially characterized and tested for copper detection. The jalpaite composition (nominally Cu₂S·3Ag₂S) is considered the best preparation for detection of copper.²⁰ Some references suggest that the jalpaite phase is unstable and undergoes loss of copper to the solution thereby causing poorer detection limits that may be calculated based on solubility. Other references suggest that this is not the case and the poorer detection depended on what phase was present. Some of the problems in preparing the jalpaite composition are the rate of addition of the reagents and the precipitation of the mixed phases. Silver sulfide appears to precipitate faster under certain circumstances than does copper sulfide, sometimes producing a mixture of phases rather than a compound. A precipitation reagent based on thiourea rather than the more frequently used sodium sulfide was initially chosen.¹² This reagent decomposes more slowly into sulfide, thereby forming a single phase rather than a mixture of phases. To prepare various phases, the ratio AgNO₃/CuSO₄ was varied in steps of 10%, higher and lower. After extensive washing and drying, the powders were pressed into pellets and tested for the detection of copper. Preliminary data show that the electrode prepared with 20% excess silver over copper performs the best. However, all the preparations are very similar to each other and to the commercial system in performance. One major difference is the conductivity of these membranes compared to the commercial electrode. These pellets all have resistances less than 100 Ohms whereas the commercial system is in the 100 KOhm range.

The membrane powders prepared by the thiourea method were characterized by powder X-ray diffraction. This study showed that the desired jalpaite phase was only partially present in a mixture of acanthite (Ag₂S) and covellite (CuS). Additionally, careful examination of the published X-ray diffraction data on similar preparations also shows that the authors had prepared mixtures rather than a pure phase of jalpaite, as was claimed.¹²

A better preparation, that matched the powder X-ray diffraction database jalpaite phase very closely, was prepared by a major modification of the procedure of Heijne²⁴ as follows: Sodium sulfide (10.3g, 42.9 mM) was dissolved in 60 mL of distilled water, cooled in ice, and stirred under argon. Copper sulfate (3.046 g, 12.2 mM) and silver nitrate (4.1 g, 24.1 mM) were dissolved in 60 mL of distilled water. Silver sulfate formed and was redissolved by dropwise addition of concentrated ammonium hydroxide. The blue solution was added dropwise to the silver sulfide solution over a 20 min. period. After the addition was complete, the black mixture was heated at about 80°C for 30 min, cooled, and filtered. The precipitate was washed with water - 3x, 50% nitric acid - 2x, water - 3x, and then dried under a vacuum.

Powder X-ray diffraction patterns for the material prepared by thiourea and sodium sulfide precipitations are shown in Figure 7. Reference spectra of jalpaite and acanthite are also given. It is interesting that to form the jalaite phase, the copper must be in excess over the silver whereas in jalpaite, the copper:silver ratio is 1:3. The best ratio used for forming this phase is 1:2. Less copper invariably forms some CuS (Figure 7). An additional issue is that in jalpaite the copper is in a +1 oxidation state and preparations normally start with copper as +2. The reduction of the copper forms oxidized sulfur (to some form), which must be removed.

Figure 7 – Powder X-Ray diffraction data for copper membrane material prepared by two techniques. For a comparison, the library spectra are shown in magenta bars under the two sample spectra. The sample in spectrum A was prepared by the thiourea reduction technique as per Neto, et al.¹² The powder X-ray diffraction data show that it is almost completely acanthite (Ag_2S). Its response characteristics are shown in Figures 2 and 3. Other preparations by this technique gave mixed sulfides with no pure phases. The sample in spectrum B was prepared by the Na_2S technique as outlined in the experimental section. All spectra were background subtracted and smoothed and only the most relevant angle range is shown.



In comparison with the commercial jalpaite electrode, some of the solid-state membrane preparations had better LODs and some worse. However, none of the electrodes prepared with numerous preparations of membrane material preformed exactly like the commercial jalpaite electrode when placed in copper buffering media (two examples are shown in Figure 3). Thus, the commercial electrode was used for the ligand experiments. Additionally, many electrodes stopped responding for some unknown reason. Even the commercial electrode stopped working and could not be regenerated by polishing. This appeared to be most problematic after exposure to Cu^{+1} solutions.

Design and Operation of the Computer-Controlled Potentiostat

Three different systems were evaluated for the computer-controlled potentiostat, which has been termed the Electrode Maker Board (EMB). The advantages and disadvantages of each are shown in Table 3. None are ideal in terms of cost, power, and functionality. For the final design, the Analog Devices ADuC812 was chosen as the best compromise. In earlier designs, the ADuC812 was also used to control and take data from the ion selective electrodes as a separate board. However, due to the increased flexibility of the TI chip and lower power, the TI-MSP430F149 was chosen for the Environmental Water Quality Monitoring System (EMS) – a separate system that controlled and took data from the ion selective electrodes. Future designs could use the EMS system to be a potentiostat and to control and read the ion selective electrodes to allow smaller instrumental packages.

Table 3 – Design parameters for the various microprocessors used.

Parameters	Microchip Various devices	Analog Devices ADuC812 (Choice for EMB)	Texas Instruments MSP430F149 (Choice for EMS)
Flash Programming	Yes - but only with circuit changes	Yes – but only with circuit changes (not reliable at 2.7V)	Yes - without hardware manipulation
Power Consumption	1-2 mA	2-3 mA	1-200 μ A
Integral Analog/Digital Converter	No	Yes - 12 bit (8) + chip temperature	Yes - 12 bit (10) + chip temperature + battery voltage
Integral Digital/Analog Converter	No Maxim 5722 used at \$4.38	Yes –12 bit (2) – However, not floatable	No Maxim 5722 used at \$4.38
Battery Monitor	No	No – requires use of one A/D channel	Yes
Crystal	Wide choice	Limited frequencies - low clock speed for low power – requires special I/O software	Can use 32768 Hz for small size
Development Software for assembly language	Good	Good	Excellent – allows on-chip debugging
Pins Available	Number of choices – many higher power consumption	Barely enough/limited functionality	Sufficient
Cost in quantities of 1000	Depends on choice (requires D/A as extra)	\$8.30	\$7.19 (requires D/A as extra)

The EMB system was modeled after ones described in the literature.²⁵ A general schematic is shown in Figure 8. The D/As and A/Ds are driven directly from the microprocessor. Any of the pins in the eight pin round plug can be selected with a Maxim MAX349 digital switch. The selection of the bias resistors, Pt or center reference, and A/Ds are made with a Maxim MAX394 digital switch. The two ground connections are made with manual jumper plugs. However, even with the plugs in place, the ground is through pins on the microprocessor. This limits the current to ground to approximately 2 mA, but allows floating these connections under software control. A picture of the card is given in Figure 9.

Figure 8 – General schematic for the Electrode Maker Board. The circular design is a receptacle that allows attachment of an electrode array. In figure 9, wires are soldered to the platinum reference and pin one of the circular array.

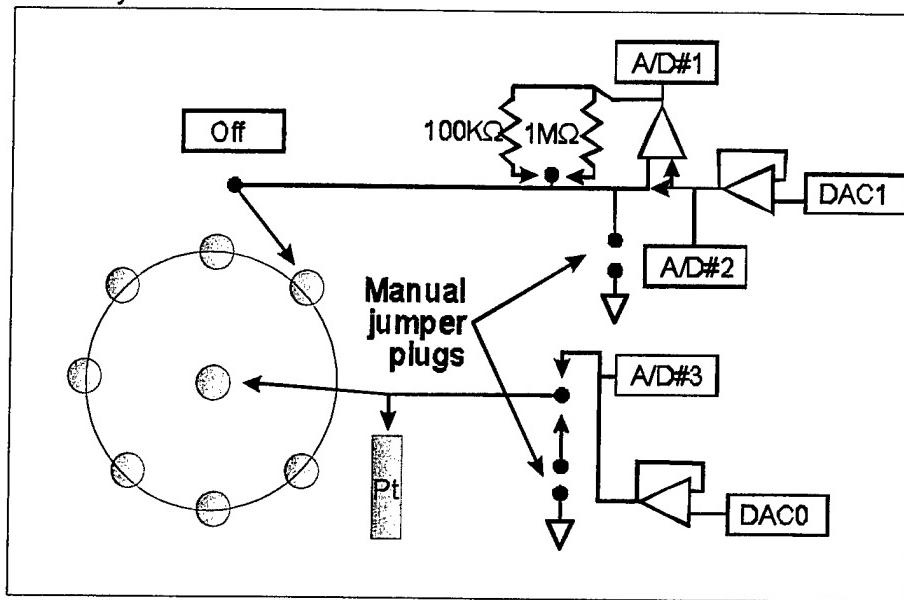
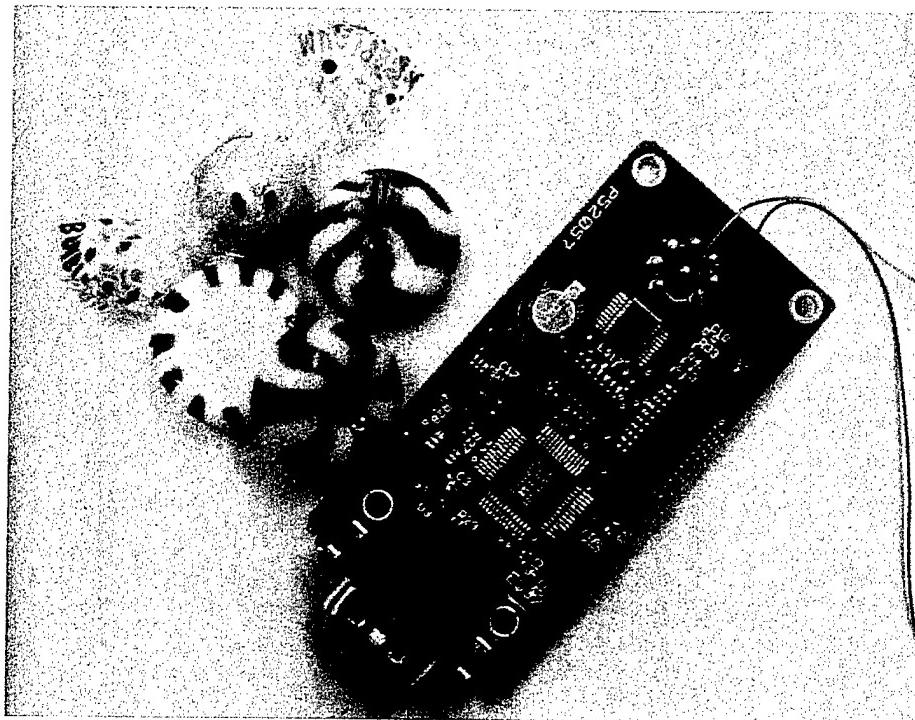


Figure 9 – Picture of the Electrode Maker Board. Candy is used to illustrate the small size of the system.

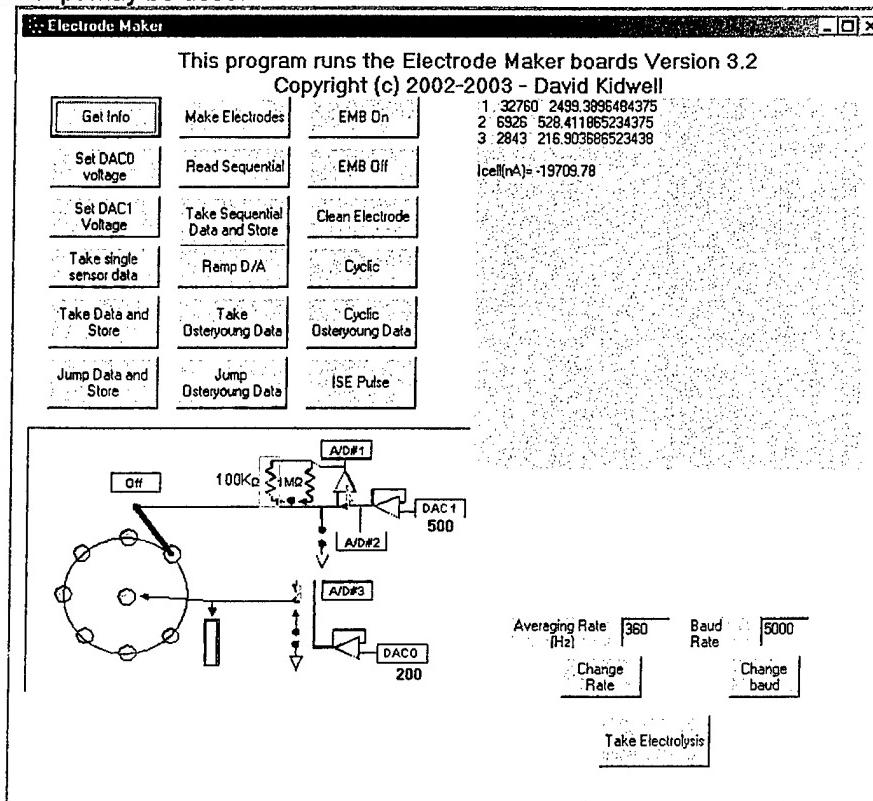


The general design considerations of the EMB were to allow several modes to be computer controlled. For example, the software can select any one of the seven working electrodes in the circular array, can select either the external platinum counter electrode or a center electrode in the array, can select the bias current for the current to voltage converter, and can select how the system is driven and grounded. Both on-board microcode and a Visual Basic interface

controlled the EMB. Because of the flexible design, the EMB can be used either as a potentiostat (controlling voltage and measuring current) for monitoring enzyme electrodes, a digital cyclic voltammetry system, which can employ numerous types of cyclic voltammetry, a constant current source (controlling voltage and measuring current), or to perform electrolysis or plating. All these modes have been confirmed with the EMB system. Additional design considerations for the EMB system are that it be powered through the RS-232 port of the computer (so low current draw is necessary) and only use positive potentials (as referenced to ground) for all the analog circuitry. The latter requirement was to allow easy transition into a battery-powered system. Because of the use of only positive potentials, biasing of the cell is necessary to allow it to swing in a negative direction relative to the bias yet still be positive relative to ground. This bias may be in any direction by the appropriate potentials generated by DAC0 and DAC1. Generally, the voltage across the cell is the difference between these two potentials. Using two DACs allows a total sweep in cyclic voltammetry of up to 5 V with a normal DAC swing of only 0-2.5V. However, the bias on the current-voltage converter will limit this range, as it cannot go negative.

For use in electrochemical generation of metal ions, the EMB was set-up as shown in Figure 10, a screen shot of the Visual Basic driver. In this case, the copper electrode was in pin 1, the external platinum electrode was used, and the voltage applied for electrolysis was 300 mV (difference between DAC1 and DAC0). One data point was taken (normally the data are taken and stored in a CSV file for import into Excel and data analysis) and the measured current was 19.7 μ A.

Figure 10 – Screen shot of EMB system when performing some electrolysis experiments. The switches are selected manually by clicking on the appropriate selection points in the schematic. Alternatively, a script may be used.

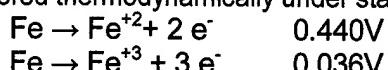


Tests of the system, shown in Figure 9, were carried out for both copper and iron. For these tests, colorimetric dyes were used to measure the amount and oxidation state of the metal ion formed in a continuous fashion and the results compared to the amount calculated from the current generated.

Production of Ferrous Ions

Ferrous and ferric ions may be produced from the oxidation of iron metal. The standard electrochemical potentials are shown in Scheme 1.

Scheme 1 – Standard electrochemical potentials for oxidation of iron. Since $\Delta G = -nFE$, the production of Fe^{+2} would be favored thermodynamically under standard conditions.

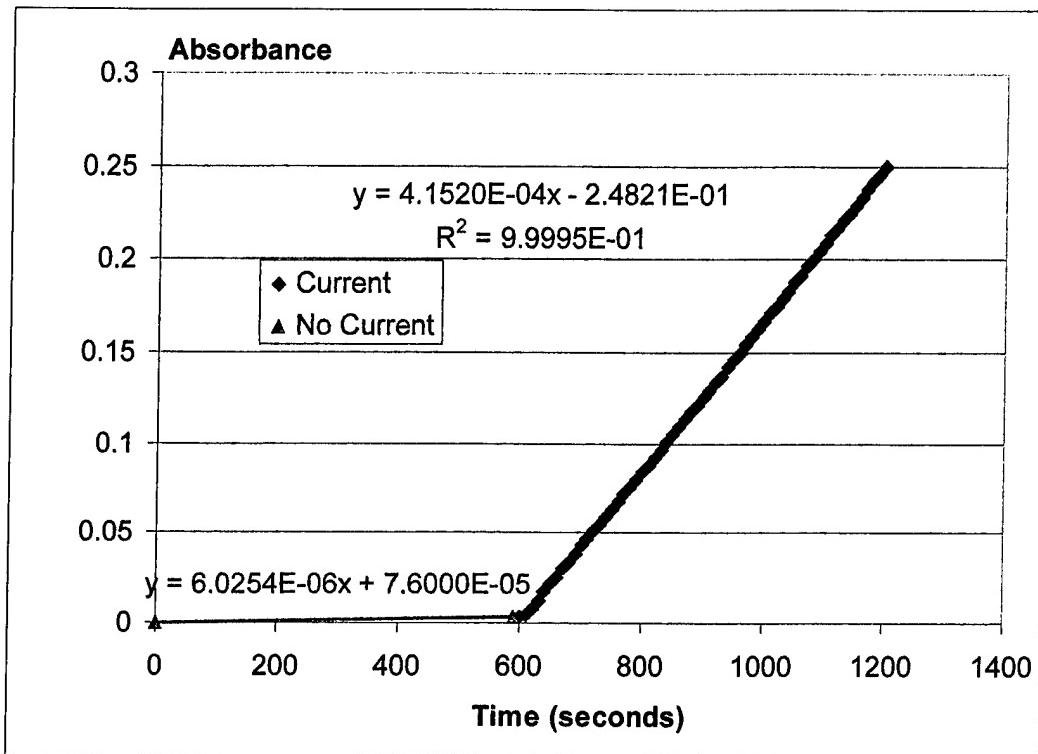


The production of ferrous iron would be favored under thermodynamic conditions when everything is in standard state. Of course, thermodynamics can tell us nothing of the rate of the two reactions and one may be favored kinetically. Additionally, in dilute solution in the presence of high salt concentrations (*i.e.* seawater – concentrations far from standard conditions), which form of iron that would be produced electrolytically is not clear. To resolve this issue, the electrolysis was carried out in a flowing system where the ion selective electrode was replaced with a spectrometer in the presence of a selective colorimetric dye. The results are shown in Figure 11. The current efficiency can be calculated from the observed rate of production of ferrous (from the slope of the absorbance line and the molar extinction coefficient) compared to the rate calculated from the measured current. The measured extinction coefficient (from ferrous sulfate standards) in 0.55M salt water was $10774 \text{ mole}^{-1}\text{cm}^{-1}$. This compares favorably to the literature value of 11,000 in water.^{26,27} The equation for efficiency was:

$$\begin{aligned} \text{Observed moles/sec} &= \text{slope of least squares fit/extinction coefficient*solution volume} \\ \text{Calculated moles/sec} &= \text{observed current}/96493/2 \\ \text{Efficiency} &= \text{Observed}/\text{Calculated}*100 \end{aligned}$$

The calculated efficiencies from two runs were 118% and 103%. The calculated efficiencies are over 100% probably due to imprecision in the current measurement. Accounting for most of the current in the production of ferrous ion indicates that most of the iron is produced as ferrous in this system. If ferric ion were being produced then the current efficiencies would be lowered, as 1,10-phenanthroline only produces significant color with ferrous ion (the measured extinction coefficient is $2234 \text{ mole}^{-1}\text{cm}^{-1}$).

Figure 11 – Monitoring of ferrous production with 1,10-phenanthroline in 0.55M NaCl, pH ca. 8 (unbuffered). The phenanthroline concentration was 0.5 g/mL (from 400 μ L of 50 mg/mL into 40 mL of saltwater). The color production was monitored at 510 nm on a HP 8451A diode array spectrometer containing a flow cell. The electrolysis took place external to the spectrometer and the solution was continuously pumped through the cell. To generate the iron, a length of #16.5 piano wire (1 mm diameter) was glued in a 6.4 mm PVC rod with epoxy. The end was polished with 400 grit wet and dry sandpaper. The counter electrode was platinum wire. The voltage applied was 600 mV and the average current was approximately 0.29 mA. The low absorbance change in the absence of an applied voltage indicates that non-specific corrosion was low. No cathodic protection was used to reduce corrosion.

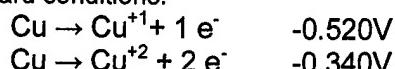


The phenanthroline will chelate the ferrous ion as it is produced and reduce the likelihood of its further oxidation to ferric ion and thereby account for the high-observed efficiencies. Additionally, it prevents precipitation of the iron as iron oxide. To test this assumption, the electrolysis was carried out in the same system but without phenanthroline present. After 1000 seconds, the phenanthroline was added and the color measured. A 39% efficiency was observed, indicating that appreciable amounts of ferric ions are produced when no chelating agent is present. However, this will be an advantage in measuring the chelating species in natural water because the chelating agent will stabilize whatever ion is most appropriate for that material. This will allow multiple ions to be monitored simultaneously. Accuracy would be reduced because some assumptions would need to be made as to the branching efficiency of the $\text{Fe}^{+2}/\text{Fe}^{+3}$ ratio in the electrolytic generation but the potential imposed could control this somewhat.

Production of Cupric and Cuprous Ions

Cupric and cuprous ions may be produced from the oxidation of copper metal. The standard electrochemical potentials are shown in Scheme 2.

Scheme 2 – Standard electrochemical potentials for oxidation of copper. In neither case will the production of copper ions be spontaneous. Since $\Delta G = -nFE$, the production of Cu^{+2} would be favored thermodynamically under standard conditions.



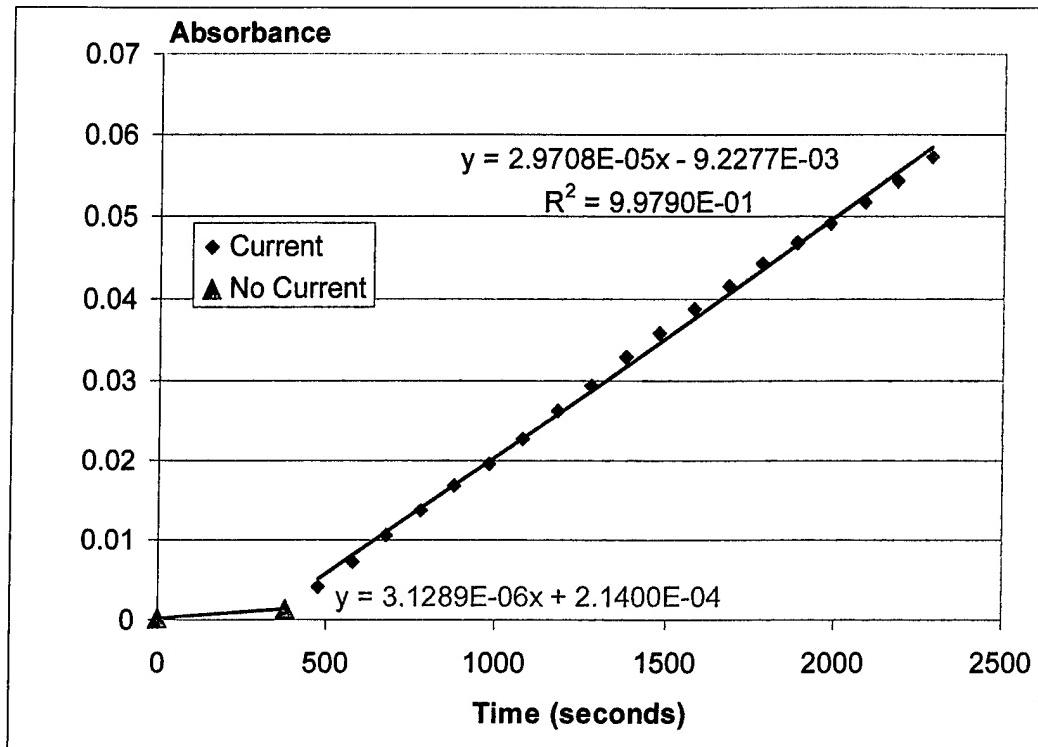
In neither case is the production of copper ions thermodynamically favored under standard conditions. However, cuprous ion is thermodynamically less stable than cupric ion and tends to disproportionate in solution. Like in the case of iron, thermodynamics can tell us nothing of the rate of the two reactions and one may be favored kinetically. Additionally, in dilute solution in the presence of high salt concentrations (*i.e.* seawater), which form of copper that would be produced electrolytically is not clear. To resolve this issue, the electrolysis was carried out in a flowing system where the ion selective electrode was replaced with a spectrometer and a selective colorimetric dye was added. The results are shown in Figure 12. The current efficiency can be calculated from the observed rate of production of cuprous (from the slope of the absorbance line and the molar extinction coefficient) compared to the rate calculated from the measured current. The measured extinction coefficient (from cupric chloride standards, dissolved in 1M HCl) in 0.55M salt water was $1757 \text{ mole}^{-1}\text{cm}^{-1}$. The equation for efficiency and calculations were performed as for the iron.

The color produced with bicinchoninic acid and cuprous ion has a substantially different λ_{\max} (558 nm) than does the cupric ion adduct (368 nm). Also, the cupric adduct has a much smaller extinction coefficient. This makes bicinchoninic acid essentially only a cupric ion detector. The current efficiency varies depending on the potential applied to the electrolysis (Table 4) but is close to 100% over a wide voltage range.

Table 4 – Observed current efficiency measured with bicinchoninic acid and varying potential.

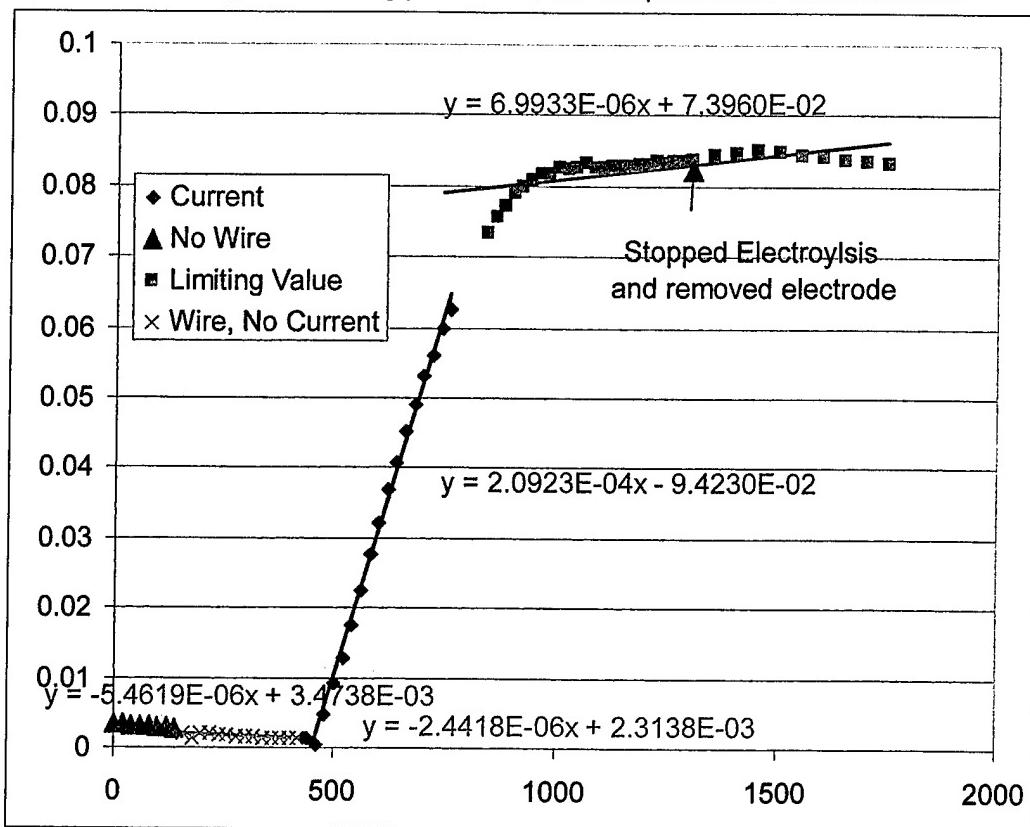
Potential (mV)	% Efficiency
400	54
600	110
800	81
1000	97
1200	87
1800	62

Figure 12 – Monitoring of cuprous production with 4,4'-dicarboxy-2,2'-biquinoline (bicinchoninic acid) in 0.55M NaCl, pH ca. 8 (unbuffered). The bicinchoninic acid concentration was 0.5 g/mL (from 400 μ L of 5 mg/mL (50:50 ethanol:water +KOH) into 40 mL of saltwater). The color production was monitored at 558 nm on a HP 8451A diode array spectrometer. A length of 18 gauge copper wire (1 mm diameter) was glued in a 6.4 mm PVC rod with epoxy. The end was polished with 400 grit wet and dry sandpaper. The counter electrode was platinum wire. The voltage applied was 600 mV and the average approximately 0.29 mA. The low absorbance change in the absence of an applied voltage indicates that non-specific corrosion was low. However, no cathodic protection was used to reduce corrosion. This run had a 90% current efficiency.



Like the iron electrolysis in the presence of phenanthroline, bicinchoninic acid may stabilize the cuprous state over the cupric state. Several additional tests were conducted with 3,5-DiBr-PAESA (4-(3,5-dibromo-2-pyridylazo-N-ethyl-N-(3-sulfopropyl) aniline) - Dojindo), which was reported to be a Cu^{+2} colorimetric agent.^{28,29} Representative results are shown in Figure 13. In a number of runs the absorbance always leveled off at an absorbance of approximately 0.1 probably because of solubility of the chelate in the salt water. A similar effect was observed upon adding standard cupric sulfate solution. From the linear part of the curve, an efficiency of 96% could be calculated, taking the extinction coefficient of the cupric complex to be $8903 \text{ mole}^{-1}\text{cm}^{-1}$. Unfortunately, 3,5-DiBr-PAESA also appears to give a similar color complex (with a similar extinction coefficient of $8000 \text{ mole}^{-1}\text{cm}^{-1}$) to cuprous ions as to cupric ions. Therefore, the exact state oxidation state of the copper ions being produced is unsettled through the use of this colorimetric detection reagent. However, the current efficiency would be half if cuprous ions were being produced rather than cupric ions. Like iron, the oxidation state produced appears to be partially determined by the chelating species present.

Figure 13 – Monitoring of cupric production with 3,5-DiBr-PAESA in 0.55M NaCl, pH ca. 8 (unbuffered). The 3,5-DiBr-PAESA concentration was 6.25 µg/mL (from 25 µL of 10 mg/mL) into 40 mL of saltwater). The color production was monitored at 622 nm on a HP 8451A diode array spectrometer. A length of 18 gauge copper wire (1 mm diameter) was glued in a 6.4 mm PVC rod with epoxy. The end was polished with 400 grit wet and dry sandpaper. The counter electrode was platinum wire. The voltage applied was 1000 mV and the average current was approximately 0.29 mA. The low absorbance change in the absence of an applied voltage (wire, no current) indicates that non-specific corrosion was low. No cathodic protection was used to reduce corrosion. This run had a 96% current efficiency if the assumed species produced was Cu⁺². The different regions were: Magenta - system drift with no wire present. This is partially due to background contamination of copper. Cyan – system drift with copper wire present. Drift is partially due to non-electrolytic corrosion of the wire and dissolution of copper oxide present from previous runs. Blue – system response during electrolysis. Green – limiting value due to insolubility of either the copper ions being produced or the complex formed in 0.55M NaCl.



Testing of system with artificial seawater

To evaluate the electrochemical generation of copper ions as a means to determine the excess ligand capacity of seawater, solutions of EDTA in various concentrations were prepared in saltwater solution (0.55M NaCl). A commercial copper electrode from Cole-Palmer was used to monitor the copper ion response as it is readily available and a miniature size is not required for this laboratory demonstration. Representative data are shown in Figure 14 and Table 5 gives the actual and measured values for EDTA by this technique. The measured values for the concentrations of EDTA correspond quite well to the expected values. For the calculated EDTA concentrations in Table 5, Cu⁺¹ is assumed to be produced. However, the binding constant of Cu⁺¹ to EDTA is not given in the literature but an estimate was made of this binding constant and is presented in Table 2. To the extent that this constant is accurate, Cu⁺¹ should compete well with Cu⁺² for the binding of EDTA. For the higher concentration of EDTA, the electrode mV

value did not return to the starting mV value. Therefore, the endpoint was chosen where the curve leveled-off. This is more practical for real samples because no sample would be available with the binding ligand present for comparison.

Figure 14 – Measuring ligand binding in salt-water solutions. A solution of 1×10^{-4} M EDTA was prepared in 0.55M NaCl. The electrode response was measured in distilled water, salt water without EDTA, and then salt water with EDTA. The large potential change from distilled water to salt water is partially due to interferences by chloride. The EDTA solution was not buffered. However, the 0.1M stock solution of EDTA, used to make the lower concentrations, was adjusted to pH 8 with HCl. The blue line shows smoothed data using a 10-point moving average. The equivalence point was chosen by eye to be the point where the electrode response returned to the level of salt water without EDTA or appeared to level off. In this case, it corresponds to a concentration of EDTA of 7.87×10^{-5} M assuming that Cu^{+1} is being produced by electrolysis. The decrease after the start of electrolysis is reproducible and of unknown origin. However, it may be due to the electrode responding to Cu^{+1} .

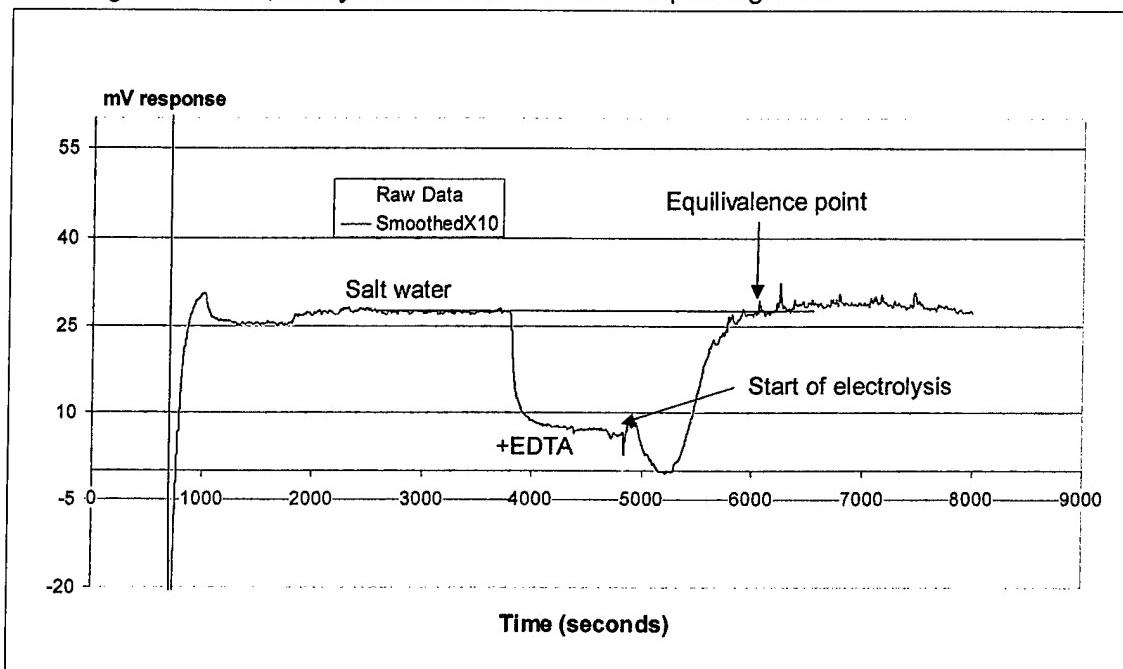


Table 5 – Expected and measured concentrations of EDTA in 0.55M NaCl. The measured values are calculated from the current-time curves assuming that Cu^{+1} is being produced.

Expected EDTA value (M)	Value measured via electrolysis (M)
1×10^{-4}	1.04×10^{-4}
1×10^{-4}	7.87×10^{-5}
1×10^{-4}	1.02×10^{-4}
2×10^{-4}	2.02×10^{-4}

These experiments demonstrate that the metal binding capacity of seawater can be easily measured by electrochemically generating a metal ion and using an ion selective electrode as a reference. If some assumptions are made,³⁰ it probably does not matter what ion selective electrode is used as only excess ligand is being measured. Therefore, with the EMB system, several metals could be in place and electrochemically generated to determine relative binding efficiencies of the natural ligands present.

Conclusions

Copper ion selective electrodes probably measure chelating affinity rather than free copper in seawater. Previous measurements gave fortuitous agreement to other measurement techniques probably due to the consistency of the environment. The toxicity of copper is likely due to the free copper present rather than the total copper. Thus, in estuaries, which have large amounts of organic matter that can complex copper, more copper can be accommodated without toxicity than in pristine water without the organic matter. A scheme for the automated measurement of binding capacity is presented. Such a system will be invaluable for monitoring metal toxicity in estuary environments. Additionally, the ease of measurement of binding ligands and their correlation to toxicity may allow regulatory agencies to be more flexible in setting standards for metals in the environment.

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- ³⁰ For the most part, it would be better to select ISEs for the species of interest and generate that metal ion. However, if one assumes that the ligands of interest also interact with copper, then a copper ISE could be used to monitor the metal ion. Only in the unlikely case where the natural ligand was many orders of magnitude different in binding of copper vs. the ion of interest would the results be inaccurate. For example, compare the calcium results in Figure 5 with the nickel results. Even though calcium has a binding constant 10⁻⁸ less than copper for EDTA (Table 2), it still competes well if at high enough concentration. Also, note that the binding constants for free copper are likely much higher than for the system modeled in Figure 2 as the copper is being removed from waters of hydration (with the free metal to form the complex) vs. from a crystal lattice. Thus, the binding constants in Table 2 should only be used as a guide for the thermodynamics of the system being modeled and measured.